ENTEROBACTER HAFNIAE

(The "Hafnia Group")

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SUMMARY. A comparative study of the biochemical reactions given by cultures of Enterobacter aerogenes and by members of the "Hafnia group" is reported. It is concluded that sufficient differences of taxonomic importance between the two warrant elevation of latter "group" from subspecies to species level. Further, it is shown that the correct name for the bacteria formerly called the "Hafnia group" or Enterobacter aerogenes subsp. alvei is Enterobacter hafniae (Moeller) Ewing.

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The following is a summation of the pertinent literature regarding the taxonomy and nomenclature of the bacteria known as the "Hafnia group."

1. According to Castellani (personal communication 1951) Bacillus asiaticus and Bacillus asiaticus var. mobilis first were described in 1905. The authors were unable to locate this publication. However, the biochemical reactions of Bacillus asiaticus were listed by Castellani (1912), by Castellani and Chalmers (1919, 1920), and in the above-mentioned correspondence. The reactions there listed were different from those given by bacteria of the "Hafnia group." At the request of the late Professor Breed, the authors examined (1951) a small collection of bacteria for Dr. Castellani. These strains were said to have been maintained in a collection at the London School of Hygiene and Tropical Medicine by a former assistant of Dr. Castellani. Two of the cultures (CDC 1378-51 and 1379-51) were labeled B. asiaticus and B. asiaticus var. mobilis, respectively. In the authors' hands both of these strains were motile and yielded bio-

chemical reactions typical of those given by the bacteria later called the "Hafnia group" (v. inf.). It was clear that the characteristics of the cultures received and examined in 1951 were not the same as those described by Castellani in the above-mentioned publications or correspondence.

1a. More important from the standpoint of nomenclature is the fact that Bacillus asiaticus Castellani is a later homonym of the validly published Bacillus asiaticus Sakharoff 1893 (Index Bergeyana, p. 120) and in consequence is illegitimate.

2. Bahr (1919) described a microorganism which he isolated from the intestinal tract of honey bees, and which he named Bacillus paratyphi alvei. This appears to be an illegitimate ternary combination since Bahr (1919) did not state that the name Bacillus paratyphi alvei was that of a subspecies. Therefore the epithet alvei was not legitimately published and Bahr's name was illegitimate in the authors' opinion.

In his description of the above-mentioned microorganism Bahr (1919) characterized it (pp. 34, 53) as indol positive, sucrose negative, and lactose negative. All studies indicate that members of the "Hafnia group" fail to produce indol, that most strains ferment sucrose, and many ferment lactose (e.g., Moeller 1954; Sakazaki 1961; Fife et al. 1965). Hence, Bahr's microorganism could not have been a member of the "Hafnia group" as constituted by Moeller in 1954 (v. inf.).

3. Kauffmann and Silberstein (1934) obtained a culture of B. paratyphi alvei (supposedly) from Dr. Bahr and concluded that it was not a member of the genus Salmonella. These investigators referred to the culture as "Paratyphus alvei" (sic) and reported that it failed to produce indol, fermented sucrose slowly, and did not utilize lactose. These facts are mentioned because of their bearing on subsequent work (v. inf.).

4. Stuart et al. (1943) characterized a collection of strains labeled biotype 32011 and included them in their second division of the genus Aerobacter. The authors (unpublished data 1955) identified these cultures as members of the "Hafnia group."

5. Gale and Epps (1943) used a microorganism (NCTC 6578, ATCC 9760) which they named Bacterium cadaveris in their work on lysine decarboxylase, as did Zittle and Eldred (1944). Moeller (1954) studied this culture (NCTC 6578) and concluded that it was a member of Hafnia (sic), but stated that it was slightly atypical. The authors and colleagues

(Fife, Ewing, and Davis 1965) included the same strain (CDC 1710-64) in their investigations and found it to be a typical member of the "Hafnia group." Gale and Epps (1943) referred to the microorganism simply as "a suitable paracolon organism Bact. cadaveris" and apparently used this specific epithet because the bacterium produced cadaverine by decarboxylation of lysine. It should be noted that Bacterium cadaveris is a later homonym of the validly published Bacterium cadaveris (Sternberg 1880) Chester 1897 (Index Bergeyana, p. 312). Hence the epithet cadaveris is illegitimate. This name also is a later homonym of Bacterium cadaveris Mez 1898 (Index Bergeyana, p. 312).

6a. Moeller (1954) included a supposedly authentic culture of "Paratyphus alvei" (sic) of Bahr in his studies and concluded that it was a member of the "Hafnia group." This strain was obtained from Prof. Kauffmann and was the same as that examined by Kauffmann and Silberstein (1934). Moeller (1954) stated that "Owing to its early isolation, this strain ought presumably to be regarded as the type species of Hafnia, under the name Hafnia alvei, for instance." The authors also examined this strain (CDC 711-54) and confirmed Moeller's conclusion that it was a member of the "Hafnia group."

6b. According to Index Bergeyana (p. 603) the name Hafnia was validly published and legitimate. The type species is listed as Hafnia alvei.

7. Sakazaki (1961) proposed that "the Hafnia should be assigned as a species, E. alvei, to genus Enterobacter Hormaeche and Edwards."

8. Ewing (1963) presented a formal nomenclatural system for the family Enterobacteriaceae, which was based upon the taxonomic system employed by Ewing and Edwards (1960). Ewing proposed that members of "Hafnia group" should be considered a subspecies (variety) of Aerobacter aerogenes, i. e., Aerobacter aerogenes subsp. hafniae, and discussed the matter in Annotation 14 of the 1963 paper. After that paper was accepted for publication, the Judicial Commission of the International Nomenclature Committee acted upon the request of Hormaeche and Edwards (1960a, 1960b) and conserved the generic name Enterobacter Hormaeche and Edwards (Opinion 28, Intl. Bull. Bact. Nomen. Tax. 13:38, 1963; see also Minute 63, *ibid.*, p. 27; par. 5, *ibid.*, p. 141; and the addendum to the paper of Ewing 1963). Thus, the nomenclature of members of the "Hafnia group" as proposed by Ewing (1963) became Enterobacter aerogenes subsp. hafniae.

Parenthetically, the Subcommittee on Enterobacteriaceae of the American Society for Microbiology voted in favor of the above-mentioned nomenclatural system and recommended its adoption by the Society and its use in the next edition of Bergey's Manual (v. Report 1964).

9. The summary of pertinent literature and relevant facts given above (par. 2, 3, 6) revealed that an error must have occurred, and that the strain labeled B. paratyphi alvei sent to Professor Kauffmann by Dr. Bahr was not an authentic culture of that species, since its biochemical reactions were not the same as those recorded by Bahr (1919). Although this particular strain was found to be a member of the "Hafnia group" by Dr. Moeller as noted in paragraph 6a (above), the selection of the specific epithet alvei obviously was incorrect. Were the members of the "Hafnia group" regarded as a genus, the generic name Hafnia (Moeller 1954) apparently would be correct.

10. Therefore, it is apparent that the epithet hafniae (par. 8, above) as employed by Ewing (1963) has priority over the earlier epithets, all of which have been proven to be illegitimate. This epithet should be used as the subspecific or specific epithet (v. inf.) in the correct name for the bacteria of the "Hafnia group."

In Annotation 14 of the above-mentioned paper (Ewing 1963) it was stated that "the bacteria in the Hafnia group are closely related to Aerobacter aerogenes and are regarded as a subspecies (variety) of that species. It is clear that sufficient differences exist between members of the Hafnia group and cultures of A. aerogenes to warrant the subspecific designations. If additional differences of taxonomic importance are found in the future, this group of bacteria may be elevated to specific rank, . . ." Analysis of data obtained during the examination of 1758 cultures that belonged to the tribe Klebsiellae (Fife, Ewing and Davis 1965), and re-evaluation of the data reported by Moeller (1954) and Sakazaki (1961), indicated that sufficient differences of taxonomic importance have been found which warrant the elevation of these bacteria from the subspecies to species level.

The biochemical reactions given by 75 cultures of Enterobacter aerogenes (Kruse) Hormaeche and Edwards and 100 strains of Enterobacter hafniae (Moeller) Ewing are given in Table 1 for comparative purposes. Further, the results of tests of particular value in the differentiation of these two species are summarized in Table 2. It will be noted (Table 2) that there are many clear-cut points of difference between

Table 1. The biochemical reactions given by cultures of Enterobacter aerogenes and Enterobacter hafniae.

Test or substrate	<u>E. aerogenes</u> (75 cul.)		<u>E. hafniae</u> (100 cul.)	
		% + (%+)*		% + (%+)*
Hydrogen sulfide (TSI agar)	-	0	-	0
Urease	-	2.7	-	3
Indol	-	0	-	0
Methyl red	37°C	0	+or-	54
	22°C	0	-	1
Voges-Proskauer	37°C	+	+or-	65
	22°C	+	+	99
Citrate (Simmons')	37°C	+	(+)or-	(58)
	22°C		d	3 (79)
KCN (growth)		+	+	96 (1)
Motility	37°C	+	+	93
Gelatin	22°C	(+)or-	-	0
Lysine decarboxylase		+	+	100
Arginine dihydrolase		-	-	9
Ornithine decarboxylase		+	+	100
Phenylalanine deaminase		-	-	0
Glucose	acid	+	+	100
	gas	+	+	100
Lactose		+	-or(+)	(23)
Sucrose		+	d	12 (61)
Mannitol		+	+	100
Dulcitol		-	-	1
Salicin		+	d	13 (8)
Adonitol	acid	+	-	0
	gas	+	-	0
Inositol	acid	+	-	0
	gas	+	-	0
Sorbitol		+	-	0
Arabinose		+	+	96
Raffinose		+	-	0
Rhamnose		+	+	93 (7)

* Figures in parentheses indicate the percentage of delayed reactions (3 days or more)

+ 90% or more positive within 1 or 2 days incubation

- 90% or more negative within 1 or 2 days incubation

(+) positive reaction after 3 or more days

+or- majority of strains positive, occasional cultures negative

-or+ majority of cultures negative, occasional strains positive

(+) or + majority of reactions delayed, some occur with 1 or 2 days

d different reactions: +, (+), -

Table 1 continued

Test or substrate	<u>E. aerogenes</u>		<u>E. hafniae</u>	
		% (%)		% (%)
Malonate	+ or -	74.7	+ or -	74
Mucate	+	94.7	-	0
Nitrite from nitrate	+	100	+	100
Organic acids*				
citrate	+	97.3	+	99.1
D-tartrate	- or +	40	-	0
Jordan's tartrate	+ or -	89.3	+ or -	75.4
Sodium alginate	-	0	-	0
Lipases				
corn oil	-	0	-	0
triacetin	-	2	-	0
tributyrin	-	0	-	0
Pectate liquefaction	-	0	-	0
DNase	-	0	-	0
Xylose	+	100	+	97
Maltose	+	100	+	100
Cellobiose acid	+	98.7	d	76 (16)
gas	+	98.7	d	67 (22)
Glycerol acid	+	100	+	100
gas	+	98.7 (1.3)	+	95 (5)
Alpha methyl glucoside	+	96 (2)	-	0
Erythritol	-	0	-	0
Esculin	+	98	-	6 (2)

*Method of Kauffmann and Petersen (1956)

Table 2. Differentiation of *E. aerogenes* and *E. hafniae*.

Test or substrate		<i>E. aerogenes</i>		<i>E. hafniae</i>	
			%+ (%+)*		%+ (%+)*
Adonitol	acid	+	98.7	-	0
	gas	+	98.7	-	0
Inositol	acid	+	100	-	0
	gas	+	100	-	0
Sorbitol		+	100	-	0
Raffinose		+	96	-	0
Salicin		+	98.7(1.3)	d	13(8)
Alpha methyl glucoside		+	96(2)	-	0
Esculin		+	98	-	6(2)
Methyl red	37°C	-	0	+or-	54
	22°C	-	0	-	1
Voges-Proskauer	37°C	+	100	+or-	65
	22°C	+	100	+	99
Citrate (Simmons')	37°C	+	93.7	(+)or-	(58)
	22°C			d	3(79)
Gelatin	22°C	(+)or-	(77.3)	-	0
Mucate		+	94.7	-	0

*Figures in parentheses indicate percentage of delayed reactions (3 days or more).

these two kinds of bacteria and additional differences, which are not as well-defined but nonetheless are helpful in differentiation.

Analysis of the data presented in the tables, led the authors to the conclusion that the "Hafnia" bacteria should be raised to the species level, but not to generic status. The correct name for these bacteria then, is *Enterobacter hafniae* (Moeller) Ewing comb. nov.

For additional information regarding the above-mentioned bacteria, as well as about the tribe *Klebsiellae* in general, the reader is referred to the publications of Fife, Ewing, and Davis (1965). One of the authors (WHE) was misled into thinking that the correct name for the bacteria of the "Hafnia group" was *Enterobacter aerogenes* subspecies (var.) *alvei* (Bahr) Moeller, and unfortunately employed this name (and *Enterobacter alvei*) in several publications (Davis and Ewing 1964; Lubin and Ewing 1964; Fife, Ewing, and Davis 1965). This error can and will be corrected in the next printing of the last publication cited. The name *Enterobacter hafniae* was employed by Ewing (1966, 1967).

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